PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/558 — A1

(11) International Publication Number: **WO 97/31268**

(43) International Publication Date: 28 August 1997 (28.08.97)

(21) International Application Number: PCT/US97/02159

(22) International Filing Date: 24 February 1997 (24.02.97)

(30) Priority Data:
08/604,776 23 February 1996 (23.02.96) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/604,776 (CIP)
Filed on 23 February 1996 (23.02.96)

(71) Applicant *(for all designated States except US)*: UNIVERSAL HEALTHWATCH, INC. [US/US]; Oakland Center, 8990-E Route 108, Columbia, MD 21045 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: BERNSTEIN, David [US/US]; 5814 Melville Road, Eldersburg, MD 21784 (US).

(74) Agents: SANDERCOCK, Colin, G. et al.; Foley & Lardner, Suite 500, 3000 K Street, N.W., Washington, DC 20007-5109 (US).

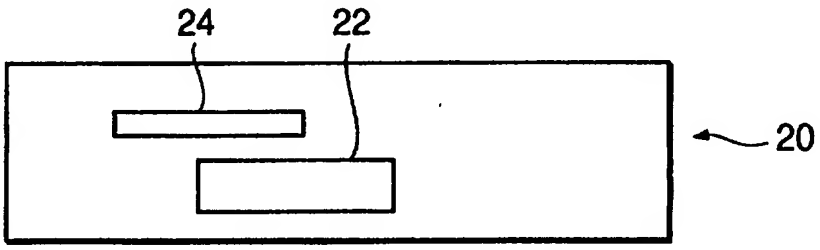
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CHROMATOGRAPHIC STRIP HAVING DETECTION AND CONTROL ZONES ORIENTED PARALLEL TO THE DIRECTION OF FLOW

(57) Abstract

Improved chromatographic strip binding assay devices are provided for determining the presence or amount of an analyte present in a sample. Assay label reagents interact with capture reagents immobilized in a testing region (22) on the strip substrate (20) to generate a visually detectable image indicative of the test result. The immobilized capture reagents responsible for the location and configuration of the test result images are applied to the strip at locations substantially parallel to the flow of reagent fluid within said strip.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

CHROMATOGRAPHIC STRIP HAVING DETECTION AND CONTROL ZONES ORIENTED PARALLEL TO THE DIRECTION OF FLOW

Field of the Invention

The present invention relates to qualitative diagnostic test strip devices incorporating a visually detectable result format which generates or develops an image to indicate a positive test result for analyte and another image to indicate a procedural control for analyte. More particularly, it relates to improved diagnostic test strip devices wherein the reagents responsible for the development of the test result and control images are oriented substantially parallel to the flow of reagent fluid within said strip.

Background of the Invention

In the development of the medical diagnostics field, there has been explosive growth in the number of substances to be detected in physiological test samples. Various analytical procedures are commonly used in diagnostic assays to determine the presence and/or amount of these substances of interest or clinical significance. These clinically significant or interesting substances are commonly referred to as analytes.

Diagnostic assays have become an indispensable means for detecting analytes in test samples, and for the most part the medical profession has used highly automated clinical laboratories and sophisticated equipment for these determinations. There is, however, an expanding need for having analytical capabilities in doctors' offices, in the home, and in the field where electricity and other semblances of a laboratory are unavailable. Together with the diagnosis of disease or physiological conditions or disorders, there is a growing need to monitor the effects of drug therapy and chronic illness, to

detect the use of drugs of abuse and to detect the presence of contaminants.

Numerous approaches have been developed toward this end, depending to varying degrees on instrumental or visual observation of the assay result. Typical of these methods are the so called "dipstick" and "flow-through" devices and methods. The dipstick generally uses a plastic strip with a reagent-containing matrix layered thereon. A test sample is applied to the device, and the presence of the analyte is indicated by a visually detectable signal such as a color-forming reaction. The flow-through device generally uses a porous material with a reagent containing matrix layered thereon or incorporated therein. Test sample is applied to and flows through the porous material, and analyte in the sample reacts with the reagent(s) to produce a detectable signal on the porous material. Although such devices have proven useful for the qualitative determination of the presence of analytes, they often require a number of manipulative steps, for example, the addition and incubation of assay reagents and the need for intermediate washing steps.

These complex devices and methods are time consuming and require the close attention of a user who, depending on the device, must time the various assay steps and in some cases measure the reagents to be added. Hochstrasser (U.S. Pat. No. 4,059,407) discloses a dipstick device which can be immersed in a biological fluid to semi quantitate analyte in the fluid. Semi quantitation of the analyte is accomplished by using a series of reagent containing pads wherein each pad in the series will produce a detectable color (i.e., a positive result) in the presence of an increasing amount of analyte. Also of interest in the area of dipstick devices are U.S. Pat. Nos. 3,802,842, 3,915,639 and 4,689,309. Deutsch et al. describe a quantitative chromatographic test strip device in U.S. Pat. Nos. 4,094,647, 4,235,601 and 4,361,537. The device comprises a material capable of transporting a solution by capillary action, i.e., wicking.

Different areas or zones in the strip contain the reagents needed to produce a detectable signal as the analyte is transported to or through such zones. The device is suited for both chemical assays and binding assays which are typified by the binding reaction between an antigen and its complementary antibody.

Many variations on the Deutsch et al. device have been disclosed. For example, Tom et al. (U.S. Pat. No. 4,366,241) disclose a bibulous strip with an immunosorbing zone to which the test sample is applied. Grubb et al. (U.S. Pat. No. 4,168,146) describe the use of a porous test strip material to which is covalently bound an antigen specific antibody. In performance of an assay, the test strip is immersed in a solution suspected of containing an antigen, and capillary migration of the solution up the test strip is allowed to occur. As the antigen moves up the test strip it binds to the immobilized antigen specific antibody. The presence of antigen is then determined by wetting the strip with a second antigen specific antibody to which a fluorescent or enzyme label is covalently bound. Quantitative testing can be achieved by measuring the length of the strip that contains bound antigen.

Weng et al. in U.S. Pat. No. 4,740,468 describe a chromatographic strip test device including an absorbent strip having an end portion for contacting a test solution. A test region is spaced from the end portion and contains a spot of immobilized antibody specific for analyte, for example. The strip also includes an unbound label capture zone including immobilized analyte analog. In use, a test solution is prepared by mixing a sample with a first specific binding pair member, for example, a labelled antibody conjugate for analyte. After incubation, the test solution contains analyte/antibody label complexes and labelled antibody conjugate which is unbound to sample analyte. The test solution is contacted to the end of the strip which causes the test solution to migrate up the strip towards the test region. As the fluid front traverses the label capture zone, any excess or unbound label in the test solution

binds to the analyte-analog at this zone and is prevented from further migration along the strip. If analyte/antibody-label complexes are present in the test solution, these complexes will continue to migrate to the test region where they are immobilized into a sandwich complex by the immobilized antibody located in this zone. Thereafter, the end portion of the strip may be contacted with other signal-system reagents which migrate up the strip to the test region and react with the label present in the sandwich complexes to generate a detectible signal. The presence or absence of a signal at the test region indicates the presence or absence of analyte in the sample.

Such devices, however, can have a number of shortcomings. The device and method require the end user to mix and incubate the test solution and to add the signal-system reagents and the test solution in the proper sequence. The device has no procedural controls built in which will verify to the end user that all of the reagents are functioning properly and that the proper steps have been followed to indicate a reliable result.

Diagnostic test strip devices are described in U.S. Patent Nos. 5,075,078 and 4,916,056, which include a test region having a capture reagent, typically an antibody, specific for sample analyte located thereat. A conjugate pad is located adjacent to the test strip at an upstream sample entry location. The conjugate pad includes labelled antibody and any other assay reagents which may be desirable or required. In accordance with these patents, the end user simply adds an amount of the sample, for example a bodily fluid such as blood, serum or urine, to a sample entry port. The sample migrates through the conjugate pad liberating and mixing sample fluids with the other assay reagents provided in the conjugate pad. The combined fluids migrate to the chromatographic strip and are drawn through the testing zone whereupon a signal may be developed.

The devices also contain an end of test region including a pH indicator dye located at the downstream end of the strip which

changes color upon contact with the test fluids to indicate that the test is over and that no further waiting is needed to obtain the test result. Moreover, these devices provide a visually-detectable result format which generates or develops the image of a plus sign, i.e. a + sign, to indicate a positive or yes result and a minus sign, i.e. a - sign, to indicate a negative or no result are also known. See, for example, FIG 1b.

In the case of the positive test result, the image formed can appear as a spot located at the leading edge of the procedural control bar spaced from a darkened line of signal along the upstream leading edge of the patient bar adjacent an ill-defined trailing shadow area. An indistinct image which does not resemble a plus sign results. This image can easily be misinterpreted as a minus sign.

Another problem arises in multi-analyte detection strip assays when specific analyte capture zones are provided as sequential and horizontal parallel lines. When one or more analytes of a complex mixture contacts a labelled binding pair member prior to contacting a specific capture zone, the timing and placement of the capture zone can effect the sensitivity of the assay. If zones are horizontally placed in sequence to the direction of flow, labelled reagents could be exhausted before the flow reaches an upstream capture zone.

Accordingly, an improved test strip device capable of consistently providing a strong, fully defined test result image which is inherently clear, leaving no room for misinterpretation of results is still desirable or required.

Summary of the Invention

The present inventors have discovered that shortcomings of the prior art devices may be overcome by orienting the procedural control area and one or more sample test areas on the test strip parallel to the flow of reagents through the porous strip. In this way, the migrating fluid will contact the control area and

test area(s) substantially equally. The user of the device can monitor the results with confidence that strong, well-defined images will be provided depending upon the presence or absence of analyte.

The test strip device includes a chromatographic strip substrate having a length and width capable of conveying fluids in a fluid flow direction generally parallel to the length of the strip. The strip includes a sample contact region in which a fluid sample and other assay reagents may be contacted with said strip. The strip also includes a testing region disposed on said strip at a downstream location from the sample contact region. The testing region includes a sample test area defined by a first capture reagent specific for the analyte being tested, immobilized on the strip testing region. A procedural control area defined by a second capture reagent specific for an assay label immobilized on the strip test region is also provided in the testing region. The control and test areas may be of any shape (e.g., a generally rectangular configuration), although substantially similar shapes are preferred.

In a preferred embodiment, the chromatographic strip substrate comprises nitrocellulose. The first capture reagent defining the sample test area comprises immobilized antibody specific for the sample analyte being determined. The second capture reagent defining the procedural control area comprises immobilized analyte. In the preferred embodiment the test strip device includes an application pad adjacent the sample contact region of the strip and in fluid flow contact therewith. The application pad receives the test sample. It comprises a porous substrate and a diffusible label. Preferably the label conjugate comprises an antibody conjugated to a colloid gold and/or colloidal selenium label material.

In accordance with an alternate embodiment, the label conjugate comprises a monoclonal antibody from a first species specific for the analyte being tested, conjugated to a labeling

substance. The second capture reagent comprises an antibody from a second species which immunologically reacts with the monoclonal antibody of the first species.

In accordance with another embodiment, the test area and the control area are equally sized and are positioned equidistantly from the sample contact region.

In yet another embodiment the test area and the control area are positioned above an absorbent pad. Fluid that moves through the strip is channeled through these areas. This embodiment can provide greater sensitivity by virtue of increasing the proportion of fluid that passes over the test area.

The chromatographic test strip devices of this invention provide sharp clearly defined test result images which unequivocally indicate a positive or negative test result. The assays are virtually self-performing in that they require the user to simply add a few drops of sample fluid to the sample entry region and wait a few minutes for an easily understood test result to be obtained. Rapid, accurate and relatively inexpensive immuno-diagnostic tests may be provided to physicians offices or to patients directly in this format.

Other objects and advantages will become apparent from the following detailed description taken in conjunction with the drawings.

Brief Description of the Drawings

FIGS. 1a and 1b are perspective views of prior art chromatographic strip test devices;

FIGS. 2a, 2b, 2c and 2d are top views of chromatographic test strip devices in accordance with this invention.

FIG. 3 is a perspective view of a chromatographic test strip device in accordance with this invention in operation.

Detailed Description of the Preferred Embodiments

Referring now to FIGS. 2a, 2b, 2c and 2d, exemplary chromatographic strip binding assay devices of the present invention are illustrated.

The devices may be constructed in accordance with well-known methods of the prior art. See, for example, U.S. Patent No. 5,075,078, which is incorporated herein by reference in its entirety. Constructions other than those of the '078 patent readily will be apparent to the skilled artisan. Accordingly, the detailed construction of such strip assays will not be repeated herein.

FIG. 2a illustrates a strip binding assay in which sample testing area **22** and procedural control area **24** are located substantially parallel to the length of strip **20**. As shown in the drawing, the areas are substantially rectangular, but most other shapes are acceptable. Also, while both areas are illustrated as substantially the same shape, they can be different shapes as shown in FIG. 2b.

In FIG. 2b, sample testing area **32** and procedural control area **34** are located substantially equidistant along the length of strip **30**. As shown in the drawing, sample testing area **32** is substantially rectangular and procedural control area **34** is substantially circular.

In FIG. 2c, sample testing areas **42** and **43**, and procedural control areas **44** and **45** are located substantially parallel to the length of strip **40**. As shown in the drawing, the sample testing areas and procedural control areas are of different shapes.

In FIG. 2d, sample test area **52** and procedural control area **54** are located substantially parallel to the length of strip **50**. Absorbent pad **56** is positioned below test area **52** and control area **54**. During use, absorbent pad **56** channels fluid through test area **52** and control area **54**. The increase in flow of

reagent is substantial, i.e. at least 50% more sample contacts the test area compared to the same strip that does not channel the flow of reagent. The channeling of flow results in improvement to sensitivity because a greater proportion of fluid that passes along the strip contacts the test area.

In each embodiment the test area is positioned parallel to the flow of reagents through the strip. By parallel is meant that the test area is longer than it is wide with respect to the direction of fluid movement during operation of the strip.

Although, as stated above, the test and control areas can be of any shape, in general the shapes should be relatively uncomplicated so as to avoid the problems of the prior art such as localized depletion of label conjugate. Hence, substantially circular or rectangular shapes will be commonly used. Advantageously, the test and control areas will be located closely together, also to avoid problems such as localized depletion of label conjugate.

As shown in FIG. 3, fluids are wicked or permitted to migrate along strip **60** parallel to the length of the strip in the direction shown by arrow a toward sample testing area **62** and procedural control area **64**. In this embodiment, the downstream end of strip **60** has a pH indicator dye **68** coated or deposited thereon. The pH indicator dye **68** changes color when contacted by migrating sample and testing fluids.

As a migrating fluid front containing an assay label conjugate moving in the direction of arrow a, and extending across the entire width of the strip passes through the testing zone **66**, portions of the fluid front contact the capture reagent present in the sample testing area **62** and procedural control area **64** at substantially the same time. This avoids the spot formation which occurred with the prior art devices as shown in FIG. 1b. Moreover, by minimizing the distance between the areas, label conjugate in the fluid front binds more uniformly to the

capture reagents across the entire distance X and distance Y without the localized conjugate depletion previously observed. This results in substantially complete, sharp and well-defined rectangular images being developed by immobilizing the assay label substantially uniformly within the entire procedural control area and the sample control area.

By way of further illustration, the test device of this invention may comprise a test for early detection of human pregnancy. In this case, the test device performs an immunoassay for detecting an elevated level of human chorionic gonadotropin (hCG), a hormone which is present in patient urine samples at elevated levels during early stages of pregnancy. In accordance with this format, the device of this invention includes a sample test area defined by an immobilized anti-Beta HCG antibody. The label conjugate employed comprises for example an anti-Beta HCG antibody conjugated to a colloidal gold and/or colloidal selenium label. A preferred assay employing colloidal gold and colloidal selenium labels is disclosed in application serial no. -------, filed December 13, 1995 (Attorney Docket No. 73294/103), the contents of which are expressly incorporated herein by reference.

The procedural control area may comprise an immobilized hCG/anti-Beta hCG complex. In accordance with the method of use, a doctor or a patient adds a few drops of patient urine sample into the sample fluid entry aperture. The sample wets the application pad, and the selenium colloid conjugate, for example, in the pad migrates with the sample fluid along the strip and through the testing region. If the sample does not contain hCG the reddish-pink colored selenium conjugate binds only to the procedural control area of the test region which forms the visible image of the area. This clearly and unequivocally indicates that no hCG is present in the sample and that the test is functioning properly.

If hCG is present in the sample, patient hCG binds to the anti-hCG antibody on the label conjugate to form a labelled analyte complex which then binds to the anti-hCG antibody in the sample test area forming a reddish-pink area therein. The visible sign clearly and unequivocally indicates that the patient is pregnant. The patient or physician can obtain a reliable diagnosis of whether the patient is pregnant or not in less than five minutes.

In accordance with an alternate embodiment of this invention, the label conjugate comprises a monoclonal antibody from a first species conjugated to the labelling substance. The procedural control area no longer includes an immobilized analyte but instead includes an immobilized antibody from a second species which is specific for the monoclonal antibody in the label conjugate from the first species. In the hCG assay example, the label conjugate may comprise a mouse monoclonal anti hCG selenium conjugate. The procedural control area capture reagent may comprise a specific anti mouse IgG antibody immobilized to the strip. In this alternate embodiment the development of a visible signal at the procedural control area indicates that the mouse anti hCG selenium conjugate has migrated past the procedural control area and been bound by the anti-mouse capture reagent.

Having described the structural details of the new and improved assay device of this invention, the discussion will turn to a description of the materials and methods for making and using the device of this invention. In use of an assay device according to the invention, a test sample, which can be a liquid which is used directly as obtained from the source, or which has been pretreated in a variety of ways so as to modify its character, is introduced to the device, for example, through an application pad. (See for example, the '078 patent discussed above.) Alternatively, other means can be used to provide a measured or unmeasured quantity of sample to the device. The sample passes through the application pad, where it contacts one

or more reagents involved in producing the assay reaction, to the chromatographic material through which the test sample will wick and in which it will encounter one or more additional reagents involved in producing the detectable signal.

Virtually any liquid sample can be used, so long as the sample has a reasonable rate of passage through the application pad and a reasonable rate of transport along the chromatographic material. The test sample can be derived from any desired source, such as a physiological fluid, for example, blood, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, mucous, synovial fluid, peritoneal fluid, amniotic fluid or the like. The fluid can be pretreated prior to use, such as preparing plasma from blood, diluting viscous fluids, or the like; methods of treatment can also involve separation, filtration, distillation, concentration, inactivation of interfering components, and the addition of reagents. Besides physiological fluids, other liquid samples such as water, food products and the like can be used. In addition, a solid can be used once it is modified to form a liquid medium.

The present invention is particularly advantageous in that it combines several elements to form a novel assay device with which a one-step assay can be performed. The novel device simplifies the assay protocols by decreasing the number of manual steps required for its use, thereby reducing the risk of errors during use. The combination of elements in the present invention also enables the use of predetermined amounts of reagents incorporated within the device, thereby avoiding the need for reagent measurements and additions by the user. Furthermore, the reagents are situated in the device in such a way as to make the assay substantially self-performing and to facilitate the detection and quantitation of the assay results.

Definitions

A "specific binding member", as used herein, is a member of a specific binding pair, i.e., two different molecules wherein one of the molecules through chemical or physical means specifically binds to the second molecule. In addition to antigen and antibody specific binding pairs, other specific binding pairs include, as examples without limitation, biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences such as the probe and capture nucleic acids used in hybridization reactions with a target nucleic acid sequence as the analyte, complementary peptide sequences, effector and receptor molecules, enzyme cofactors and enzymes, enzyme inhibitors and enzymes, enzyme substrates and enzymes, a peptide sequence and an antibody specific for the sequence or the entire protein, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding member, for example an analyte-analog. If the specific binding member is an immunoreactant it can be, for example, an antibody, antigen, hapten, or complex thereof, and if an antibody is used, it can be a monoclonal or polyclonal antibody, a recombinant protein or antibody, a mixture(s) or fragment(s) thereof, as well as a mixture of an antibody and other specific binding members. The details of the preparation of such antibodies and their suitability for use as specific binding members are well known to those skilled in the art. When an immunoreactive specific binding member is attached to the chromatographic material of the present invention, the device is referred to as an "immunochromatograph", and the corresponding method of analysis is referred to as "immunochromatography".

Immunochromatography, as used herein, encompasses both sandwich and competitive immunoassay techniques.

An "analyte", as used herein, is the compound or composition to be detected or measured in the test sample. In a binding assay, the analyte will have at least one epitope or binding site for which there exists a naturally occurring, complementary

specific binding member or for which a specific binding member can be prepared. "Analyte" also includes any antigenic substances, haptens, antibodies, and combinations thereof. The analyte of interest in an assay can be, for example, a protein, a peptide, an amino acid, a nucleic acid, a hormone, a steroid, a vitamin, a pathogenic microorganism for which polyclonal and/or monoclonal antibodies can be produced, a natural or synthetic chemical substance, a contaminant, a drug including those administered for therapeutic purposes as well as those administered for illicit purposes, and metabolites of or antibodies to any of the above substances. Examples of the hormones which are suitable as analytes for this invention are the following: thyroid stimulating hormone (TSH), human chorionic gonadotropin (hCG), luteinizing hormone (LH) and follicle stimulating hormone (FSH). An especially preferred hormone analyte in pregnancy testing is hCG. Pathogenic microorganisms suitable for analysis by the present invention include those microorganisms disclosed in U.S. Pat. No. 4,366,241, which is herein incorporated by reference. Illustrative of some of these microorganisms are those associated with urinary tract infections, such as Streptococcus pyogenes, Streptococcus salivarus, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Proteus mirabilis and the like. The microorganisms, when assayed by the present invention, may be intact, lysed, ground or otherwise fragmented and the resulting composition or portion thereof assayed. Preferably, the microorganisms are assayed intact.

The term "analyte-analog", as used herein, refers to a substance which cross-reacts with an analyte-specific binding member, although it may do so to a greater or a lesser extent than does the analyte itself. The analyte-analog can include a modified analyte as well as a fragmented or synthetic portion of the analyte molecule so long as the analyte-analog has at least one epitopic site in common with the analyte of interest.

"Label", as used herein, is any substance which is attached to a specific binding member and which is capable of producing a signal that is detectable by visual or instrumental means. Various suitable labels for use in the present invention can include chromogens, catalysts, fluorescent compounds, chemiluminescent compounds, radioactive labels, direct visual labels including colloidal metallic and non-metallic particles, dye particles, enzymes or substrates, or organic polymer latex particles, liposomes or other vesicles containing signal producing substances, and the like. A large number of enzymes suitable for use as labels are disclosed in U.S. Pat. No. 4,275,149, columns 19-23, herein incorporated by reference. A particularly preferred enzyme/substrate signal producing system useful in the present invention is the enzyme alkaline phosphatase wherein the substrate used is nitro blue tetrazolium 5-bromo-4-chloro-3-indolyl phosphate or a derivative or analog thereof. In an alternative signal producing system, the label can be a fluorescent compound where no enzymatic manipulation of the label is required to produce a detectable signal. Fluorescent molecules such as fluorescein, phycobiliprotein, rhodamine and their derivatives and analogs are suitable for use as labels in this reaction. In an especially preferred embodiment, a visually detectable, colored particle can be used as the label component of the indicator reagent, thereby providing for a direct colored readout of the presence of concentration of the analyte in the sample without the need for further signal producing reagents. Materials for use as the colored particles are colloidal metals, such as gold, and dye particles as disclosed in U.S. Pat. Nos. 4,313,734 and 4,373,932. The preparation and use of non-metallic colloids, such as colloidal selenium particles, are disclosed in U.S. Pat. No. 4,954,452. Organic polymer latex particles for use as labels are known.

A "signal producing component", as used herein, refers to any substance capable of reacting with another assay reagent or the analyte to produce a reaction product or signal that

indicates the presence of the analyte and that is detectable by visual or instrumental means.

"Signal production system", as used herein, refers to the group of assay reagents that are needed to produce the desired reaction product or signal. For example, one or more signal producing components can be used to react with a label and generate the detectable signal, i.e., when the label is an enzyme, amplification of the detectable signal is obtained by reacting the enzyme with one or more substrates or additional enzymes to produce a detectable reaction product.

An "ancillary specific binding member", as used herein, refers to any member of a specific binding pair which is used in the assay in addition to the specific binding members of the capture reagent and the indicator reagent and which becomes a part of the final binding complex. One or more ancillary specific binding members can be used in an assay. For example, an ancillary specific binding member can be capable of binding the analyte, as well as a second specific binding member to which the analyte itself could not attach.

Reagents and Materials

A. Binding Assay Reagents

In the present invention, binding assays involve the specific binding of the analyte and/or an indicator reagent (comprising a label attached to a specific binding member) to a capture reagent (comprising a second specific binding member) which immobilizes the analyte and/or indicator reagent on a chromatographic material or which at least slows the migration of the analyte or indicator reagent through the chromatographic material. The label, as described above, enables the indicator reagent to produce a detectable signal that is related to the amount of analyte in the test sample. The specific binding member component of the indicator reagent enables the indirect

binding of the label to the analyte, to an ancillary specific binding member or to the capture reagent.

The selection of a particular label is not critical, but the label will be capable of generating a detectable signal either by itself, such as a visually detectable signal generated by colored organic polymer latex particles, or in conjunction with one or more additional signal producing components, such as an enzyme/substrate signal producing system. A variety of different indicator reagents can be formed by varying either the label or the specific binding member, it will be appreciated by one skilled in the art that the choice involves consideration of the analyte to be detected and the desired means of detection.

The capture reagent, in a binding assay, is used to facilitate the observation of the detectable signal of substantially separating the analyte and/or the indicator reagent from other assay reagents and the remaining components of the test sample. The capture reagent of the present invention is a specific binding member, such as those described above. In a binding assay, the capture reagent is immobilized on the chromatographic material to form a "capture situs", i.e., that region of the chromatographic material having one or more capture reagents non-diffusively attached thereto.

B. Application Pad

The application pad, if used in the assay, is in fluid flow contact with one end of the chromatographic material, referred to as the proximal end, such that the test sample can pass or migrate from the application pad to the chromatographic material; fluid flow contact can include physical contact of the application pad to the chromatographic material as well as the separation of the pad from the chromatographic strip by an intervening space or additional material which still allows fluid flow between the pad and the strip.

Substantially all of the application pad can overlap the chromatographic material to enable the test sample to pass through substantially any part of the application pad to the proximal end of the strip of chromatographic material. Alternatively, only a portion of the application pad might be in fluid flow contact with the chromatographic material. The application pad can be any material which can transfer the test sample to the chromatographic material and which can absorb a volume of test sample that is equal to or greater than the total volume capacity of the chromatographic material.

Materials preferred for use in the application pad include nitrocellulose, porous polyethylene frit or pads and glass fiber filter paper. The material must also be chosen for its compatibility with the analyte and assay reagents, for example, glass fiber filter paper was found to be the preferred application pad material for use in a human chorionic gonadotropin (hCG) assay device.

In addition, the application pad contains one or more assay reagents either diffusively or non-diffusively attached thereto. Reagents which can be contained in the application pad include, but are not limited to, indicator reagents, ancillary specific binding members, and any signal producing system components needed to produce a detectable signal. For example, in a binding assay it is preferred that an indicator reagent be diffusively attached to the application pad; this eliminates the need to combine test sample and indicator reagent prior to use in an assay. The isolation of assay reagents in the application pad also keeps interactive reagents separate and facilitates the manufacturing process.

Thus, if the application pad is not used, the assay reagents can be introduced in other ways, for example, by immobilization onto the chromatographic strip.

The application pad receives the test sample, and the wetting of the application pad by the sample will perform at least two functions. First, it will dissolve or reconstitute a predetermined amount of reagent contained by the pad. Secondly, it will initiate the transfer of both the test sample and the freshly dissolved reagent to the chromatographic material. In some instances, the application pad serves a third function as both an initial mixing site and a reaction site for the test sample and reagent.

In an alternative embodiment of the present invention, the reagent containing application pad is dried or lyophilized to increase the shelf-life of the device. Lyophilized application pads were found to produce stronger signals than air dried application pads, and the lyophilized application pads maintained stability for longer periods. The reagents contained in the application pad are rehydrated with the addition of test sample to the pad.

In another embodiment, the present invention can be further modified by the addition of a filtration means. The filtration means can be a separate material placed above the application pad or between the application pad and the chromatographic material, or the material of the application pad itself can be chosen for its filtration capabilities. The filtration means can include any filter or trapping device used to remove particles above a certain size from the test sample. For example, the filter means can be used to remove red blood cells from a sample of whole blood, such that plasma is the fluid received by the application pad and transferred to the chromatographic material. Such filter means are disclosed by U.S. Pat. No. 4,477,575 and WO Application No. 86/02192, published April 23, 1987.

A still further modification of the present invention involves the use of an additional layer or layers of porous material placed between the application pad and the

chromatographic material or overlaying the application pad. Such an additional pad or layer can serve as a means to control the rate of flow of the test sample from the application pad to the chromatographic material. Such flow regulation is preferred when an extended incubation period is desired for the reaction of the test sample and the reagent(s) in the application pad. Alternatively, such a layer can contain an additional assay reagent(s) which is preferably isolated from the application pad reagents until the test sample is added, or it can serve to prevent unreacted assay reagents from passing to the chromatographic material.

When small quantities of non-aqueous or viscous test samples are applied to the application pad, it may be necessary to employ a wicking solution, preferably a buffered wicking solution, to carry the reagent(s) and test sample from the application pad and through the chromatographic material. When an aqueous test sample is used, a wicking solution generally is not necessary but can be used to improve flow characteristics or adjust the pH of the test sample. In immunochromatography, the wicking solution typically has a pH range from about 5.5 to about 10.5, and more preferably from about 6.5 to about 9.5. The pH is selected to maintain a significant level of binding affinity between the specific binding members in a binding assay.

When the label component of the indicator reagent is an enzyme, however, the pH also must be selected to maintain significant enzyme activity for color development in enzymatic signal production systems. Illustrative buffers include phosphate, carbonate, barbital, diethylamine, tris, 2-amino-2-methyl-1 -propanol and the like. The wicking solution and the test sample can be combined prior to contacting the application pad or they can be contacted to the application pad sequentially. Further detailed information relating to the application pads may be found in the prior art.

C. Chromatographic Material

The chromatographic material of the assay device of the present invention can be any suitably absorbent, porous or capillary possessing material through which a solution containing the analyte can be transported by a wicking action. Natural, synthetic, or naturally occurring materials that are synthetically modified, can be used as the chromatographic material including, but not limited to: cellulose materials such as paper, cellulose, and cellulose derivatives such as cellulose acetate and nitrocellulose; fiberglass; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon); porous gels such as silica gel, agarose, dextran, and gelatin; porous fibrous matrixes; starch based materials, such as Sephadex(r) brand cross-linked dextran chains; ceramic materials; films of polyvinyl chloride and combinations of polyvinyl chloride-silica; and the like.

The chromatographic material should not interfere with the production of a detectable signal. The chromatographic material should have a reasonable inherent strength, or strength can be provided by means of a supplemental support. A preferred chromatographic material is nitrocellulose. When nitrocellulose is used, however, the material of the application pad should be chosen for its ability to premix the test sample and the first reagent, i.e., fluid-flow through a nitrocellulose membrane is laminar and does not provide the more turbulent flow characteristics which allows the initial mixing of test sample and application pad reagents within the chromatographic material. If nitrocellulose is used as the chromatographic material, then Porex(r) hydrophilic polyethylene frit or glass fiber filter paper are appropriate application pad materials because they enable the mixing and reaction of the test sample and application pad reagents within the application pad and before transfer to the chromatographic material.

A preferred chromatographic material is glass fiber filter paper. Especially preferred for use as the chromatographic

material are nitrocellulose laminated strips described in the prior art. The particular dimensions of the chromatographic material will be a matter of convenience, depending upon the size of the test sample involved, the assay protocol, the means for detecting and measuring the signal, and the like. For example, the dimensions may be chosen to regulate the rate of fluid migration as well as the amount of test sample to be imbibed by the chromatographic material.

As discussed above, in a binding assay the sample test area and procedural control area can be formed by directly or indirectly attaching their respective capture reagent to the chromatographic material. Direct attachment methods include adsorption, absorption and covalent binding such as by use of (i) a cyanogen halide, e.g., cyanogen bromide or (ii) by use of glutaraldehyde. Depending on the assay, it may be preferred, however, to retain or immobilize the desired reagent on the chromatographic material indirectly through the use of insoluble microparticles to which the reagent has been attached.

The means of attaching a reagent to the microparticles encompasses both covalent and non-covalent means, that is adhered, absorbed or adsorbed. It is preferred that capture reagents be attached to the microparticles by covalent means. By "retained and immobilized" is meant that the particles, once on the chromato-graphic material, are not capable of substantial movement to positions elsewhere within the material. The particles can be selected by one skilled in the art from any suitable type of particulate material composed of polystyrene, polymethylacrylate, polyacrylamide, polypropylene, latex, polytetrafluoro-ethylene, polyacrylonitrile, polycarbonate, glass or similar materials.

The size of the particles is not critical, although generally it is preferred that the average diameter of the particles be smaller than the average pore or capillary size of the chromatographic material. The capture reagent(s), signal

producing component(s) or reagent coated microparticles can be deposited singly or in various combinations on or in the chromatographic material in a variety of configurations to produce different detection or measurement formats. For example, a reagent can be deposited as a discrete situs having an area substantially smaller than that of the entire chromatographic material.

It is also within the scope of this invention to have a reagent, at the downstream or distal end of the chromatographic material, which indicates the completion of a binding assay (i.e., end of assay indicator) by changing color upon contact with the test solution, wicking solution or a signal producing component. Reagents which would change color upon contact with a test solution containing water are the dehydrated transition metal salts, such as CuSO sub 4, Co(NO) sub 3) sub 2, and the like. The pH indicator dyes can also be selected to respond to the pH of the buffered wicking solution. For example, phenolphthalein changes from clear to intense pink upon contact with a wicking solution having a pH range between 8.0-10.0, which is a common pH range for the assay fluids.

Reagents can be added directly to either the application pad or the chromatographic material during the performance of the assay. The preferred embodiment of the invention, however, involves the incorporation of all necessary assay reagents into the assay device so that only a liquid test sample need be contacted to the application pad to perform the assay. Therefore, one or more assay reagents can be present in either or both the application pad or chromatographic material of the present invention. The present invention further provides kits for carrying out binding assays. For example, a kit according to the present invention can comprise the assay device with its incorporated reagents as well as a wicking solution and/or test sample pretreatment reagents as described above. Other assay components known to those skilled in the art, such as buffers,

stabilizers, detergents, bacteria inhibiting agents and the like can also be present in the assay device and wicking solution.

In a sandwich binding assay, the migrating test solution or test sample contains both the dissolved indicator reagent from the application pad and the analyte from the test sample. Accordingly, both the indicator reagent and analyte are carried downstream by the advancing liquid front. Moreover, during their migration, the indicator reagent can bind to the analyte to form an indicator reagent/analyte complex. As the wicking liquid transports the indicator reagent/analyte complex through the chromatographic material, the immobilized capture reagent also binds to the analyte to render the indicator reagent/analyte complex immobilized.

Thus, the indicator reagent/analyte complex is able to advance only as long as capture reagent binding sites on the chromatographic material are already occupied and no longer available for further binding. Consequently, the greater the concentration of analyte in the test sample, the further the distal migration of the indicator reagent/analyte complex through the chromatographic material even beyond the testing region.

Any further details needed regarding the materials, reagents and methods for making them are amply provided in the above-mentioned cited patent document or are generally known by those skilled in this art. Each of the above-cited patents and pending patent applications, in its entirety, is specifically incorporated herein by reference.

Although the present invention has been described with reference to certain preferred embodiments, modifications or changes may be made therein by those skilled in this art without departing from the scope or spirit of the present invention, as defined by the appended claims.

What is claimed is:

1. In a chromatographic strip binding assay device, for detecting the presence or amount of an analyte in a sample, wherein a visually detectable sign develops to indicate a positive test result or a visually detectable sign develops to indicate a negative test result, the device comprising:

a chromatographic strip having a length and narrow width capable of conveying fluids in a fluid flow direction generally parallel to the length of said strip, said strip comprising

a sample contact region in which a fluid sample and other assay reagents may be contacted with said strip, and

at least one testing region disposed on said strip at a downstream location from said sample contact region, said at least one testing region comprising

at least one sample test area defined by a first capture reagent, specific for the analyte, immobilized on said strip, and

at least one procedural control area defined by a second capture reagent, specific for an assay label, immobilized on said strip, said test area and said procedural control area being located substantially parallel to the flow of reagents through said strip.

2. A device as in claim 1, wherein the at least one sample area and at least one control area are substantially equidistant along the length of said strip.

3. A device as in claim 1, wherein said chromatographic strip substantially channels flow of reagent through said sample test area.

4. A device as in claim 1, wherein said first capture reagent comprises a specific binding pair member for analyte.

5. A device as in claim 4, wherein said first capture reagent is selected from the group comprising an antigen or an antibody.

6. A device as in claim 1, wherein said second capture reagent comprises a specific binding pair member for said assay label.

7. A device as in claim 6, wherein said second capture reagent is selected from the group comprising an antigen or an antibody.

8. A device as in claim 1, further comprising a housing for surrounding and holding said strip, said housing comprising

a sample entry aperture disposed adjacent said sample contact region of said strip, and a test result window disposed adjacent said testing region of said strip.

9. A device as in claim 1, further comprising a conjugate pad comprising a porous pad substrate, having said assay label therein, disposed adjacent said strip at said sample contact region.

10. A device as in claim 9, wherein said first capture reagent comprises an antibody specific for the analyte, said second capture reagent comprises immobilized analyte, and said assay label in said conjugate pad comprises an antibody, specific for the analyte, conjugated to a labelling substance.

11. A device as in claim 10, wherein said labelling substance comprises colloidal selenium, colloidal gold, or both.

12. A device as in claim 10, wherein said second capture reagent comprises an immobilized antibody/analyte complex.

13. A device as in claim 9, wherein said first capture reagent comprises an antibody specific for the analyte, said second capture reagent comprises an antibody specific for said assay label, and said assay label comprises an antibody, specific for the analyte, conjugated to a labelling substance.

14. A device as in claim 13, wherein said labelling substance comprises colloidal selenium, colloidal gold, or both.

15. A device as in claim 13, wherein said assay label comprises a monoclonal antibody from a first species specific for the analyte being tested for and said second capture reagent comprises an antibody from a second species specific for the monoclonal antibody of the first species.

16. A device as in claim 15, wherein said assay label comprises a mouse monoclonal antibody specific for the analyte, and said second capture reagent comprises an anti-mouse IgG antibody.

17. A device as in claim 1, further comprising an end of test indicator region on said strip at a downstream location from said testing region and defined by an indicator reagent capable of generating a visually detectable signal upon contact with testing fluids to indicate that fluids have flowed to said end of test indicator region and that the test result indicated at said testing region is a final test result.

18. A device as in claim 1, comprising more than one sample test area, more than one procedural control area, or both.

19. A diagnostic test kit comprising the device of claim 1.

FIG. 1a

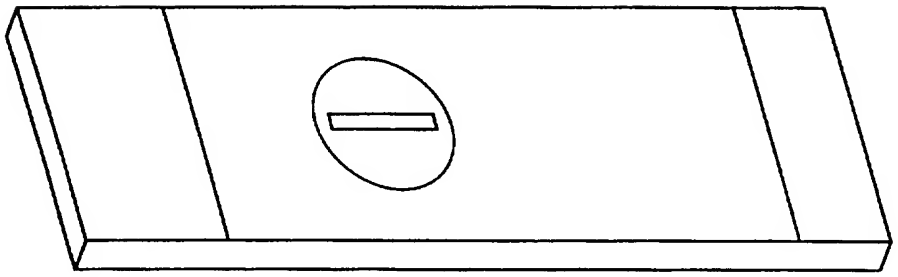


FIG. 1b

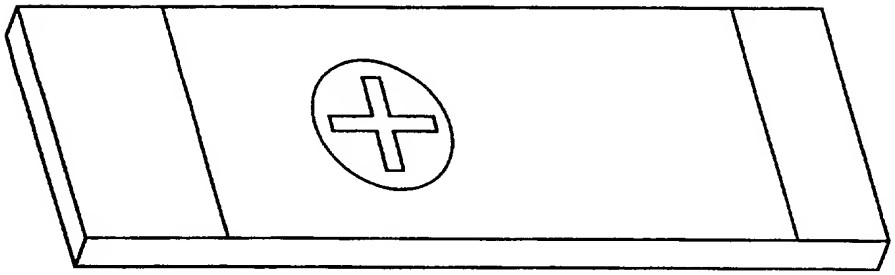


FIG. 2a

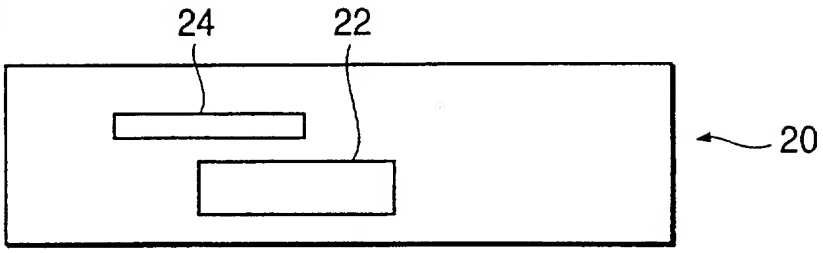


FIG. 2b

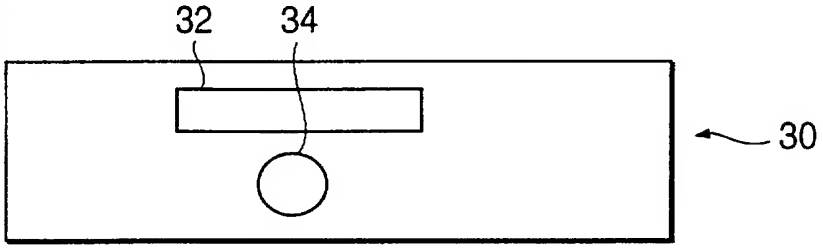


FIG. 2c

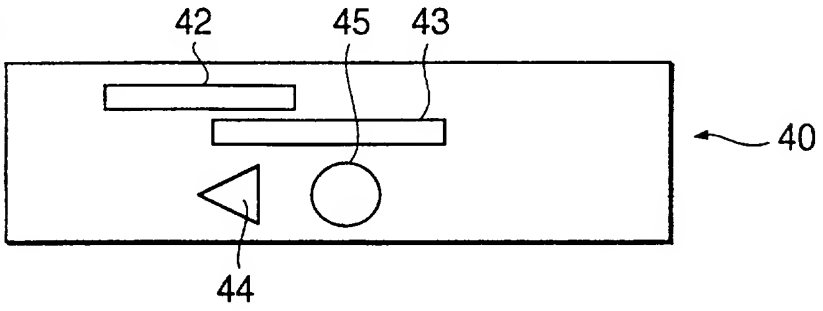


FIG. 2d

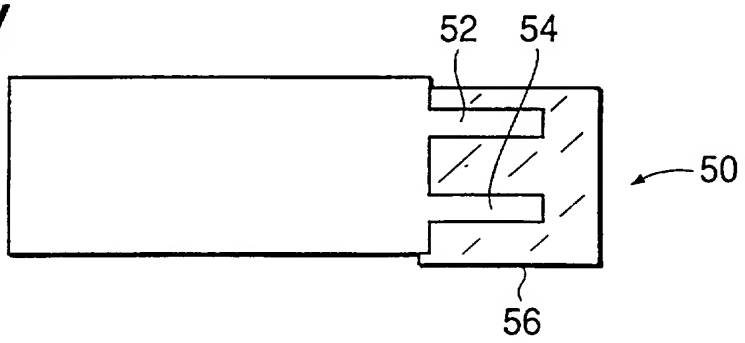
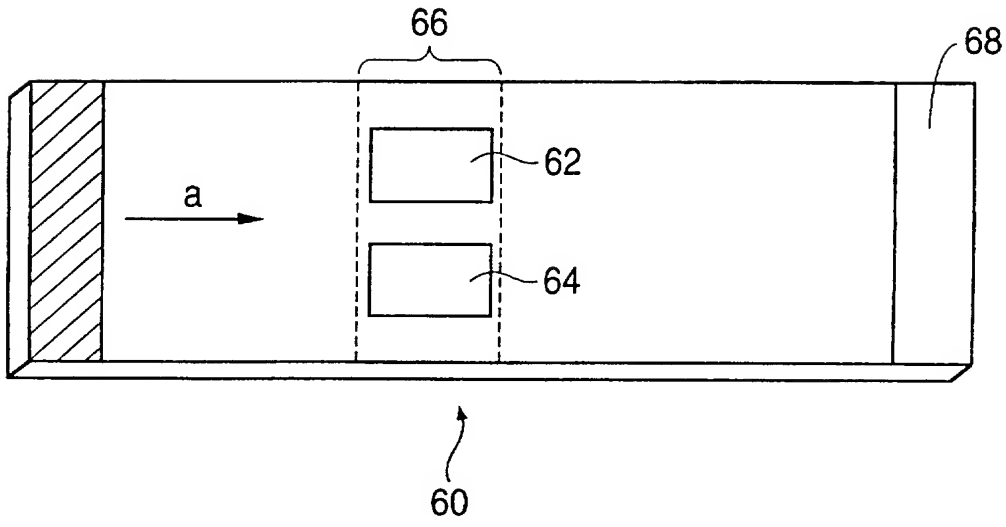


FIG. 3



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02159

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/558
US CL :436/514; 422/56

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 5,075,078 A (OSIKOWICZ et al) 24 December 1991, see entire document.	1-18 ------- 19
X ---- Y	US 4,916,056 A (BROWN, III et al) 10 April 1990, see entire document.	1-18 ------- 19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 08 JUNE 1997	Date of mailing of the international search report 16 JUL 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHRISTOPHER CHIN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02159

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

422/55, 56, 57, 58, 61;
435/7.92, 7.94, 287.1, 287.2, 287.7, 287.9, 805, 810, 967, 970, 975;
436/164, 169, 510, 514, 518, 525, 530, 531, 805, 810, 814